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Cell Cycle Synchrony Unmasks the Influence of p53 Function on Radiosensitivity of Human Glioblastoma Cells

Garret L. Yount, Daphne A. Haas-Kogan, Charlie A. Vidair, Martin Haas, William C. Dewey, and Mark A. Israel

Abstract

Although ionizing radiation causes DNA damage that can play a role in tumorigenesis, such irradiation is also an important modality of cancer therapy. We studied the radiation response of the U-87 MG human glioblastoma cell line and transfected derivatives in which p53 function had been inactivated. Although little effect of p53 on the radiation sensitivity of asynchronously growing cultures could be detected, inactivation of p53 resulted in a large increase in clonogenic survival when cells synchronized by mitotic selection were irradiated in early $G_1$. The radiation dose sufficient to reduce cellular clonogenicity by 1 log in cells expressing functional p53 was $3.26 \pm 0.12$ Gy, whereas a much higher dose ($7.41 \pm 0.44$ Gy) was required to achieve the same killing effect in cells in which p53 was inactivated. Apoptosis was excluded as a probable mechanism contributing to the radiosensitivity of these cells.

Introduction

Radiation-induced DNA damage and cytotoxicity are central issues for understanding tumorigenesis and for optimizing radiation therapy, an important modality in cancer therapy. The evaluation of radiation-induced cell death has been dominated by extensive experimental analyses demonstrating the ability of radiation to stop cells from replicating (1, 2). Such radiation-induced reproductive failure is closely associated with the cellular loss of genetic material and the aberrant segregation of chromosomes that occurs after the exposure of cells to radiation (3). More recently, a second cellular response to irradiation, apoptosis, has been recognized to contribute to the sensitivity of various cell types to irradiation (4). Apoptosis is a mechanism of active cell death that is characterized by a rapid loss of plasma membrane integrity after DNA fragmentation and requires the expression of numerous cellular genes (5).

The p53 gene encodes a transcription factor that contributes to several different cellular activities, including apoptosis, and the ability of cells to express a functional p53 protein may be a determinant of cellular response to radiation (6). p53 plays an important role in the induction of apoptosis by agents that cause DNA-strand breakage, such as radiation (4). In addition to the importance of p53 in mediating apoptosis, the ability of p53 to regulate the passage of cells through the cell cycle may contribute to the effect of p53 on the sensitivity of cells to irradiation. Most mammalian cells exhibit transient delays in $G_1$ and $G_2$ after irradiation (7). The activation of these cell cycle checkpoints may affect radiosensitivity by providing time for repair of radiation-induced DNA damage before the initiation of cell death (8). p53 is required for the $G_1$ arrest after ionizing radiation, and cells having mutant or no p53 genes fail to exhibit this response (9, 10).

We investigated the influence of p53 function on the radiosensitivity of the U-87 MG cell line originating from glioblastoma multiforme, a rapidly growing and highly invasive human brain tumor typically treated by therapeutic irradiation. U-87 MG cells express wild-type p53 (11) and display alterations in their progression through the cell cycle in response to radiation treatment (12). We inactivated wild-type p53 function in U-87 MG cells by introducing a dominant negative mutant p53 gene. We found that inactivation of p53 results in a significant decrease in radiosensitivity that may result from loss of a prolonged $G_1$ arrest, which was observed only in tumor cells expressing a functional wild-type p53.

Materials and Methods

Cell Culture, Synchronization, and Radiation Treatment. U-87 MG cells were acquired and maintained as described previously (13). All cells were grown in DMEM containing 10% fetal bovine serum (GIBCO-BRL, Grand Island, NY) supplemented with penicillin and streptomycin. All experiments were performed on exponentially growing tumor cells (doubling time, approximately 48 h) at a density between 9 and 45 cells/mm². Irradiation of asynchronous cultures (Figs. 1 and 2A) was performed at room temperature using a 150 kvp Philips X-ray machine without any filter at a dose rate of 1.2 Gy/min. Synchronized populations (93–96% in mitosis) were obtained by shaking mitotic cells from asynchronous cultures grown in plastic roller bottles as described previously (14). Synchronized mitotic cells were immediately cooled to 4°C, pelleted by centrifugation at 128 $\times$ g, and frozen viably for long-term storage (15). Labeling index experiments confirmed that U-87 MG cells frozen after mitotic selection and replated progressed through the $G_2$-$S$ phase transition along the same time course as cells plated immediately after selection without freezing (data not shown). Irradiation of synchronous cultures (Figs. 2B, 3, and 4) was performed on ice using a Westinghouse QUADROCONDEX X-ray machine without any filter at 250 kvp and 15 mA at a dose rate of 2.8 Gy/min. Irradiation of cultures depicted in Fig. 2C was performed on a Philips X-ray machine as described above.

Retroviral Vector Construction and Retrovirus Infection. The construction of recombinant retroviruses encoding either the LUX3 gene or a mutant p53 cDNA under the control of the Moloney MuLV long terminal repeat has been described previously (16). The mutant p53 retrovirus encoded a p53 cDNA mutated at codon 175 (17), which was originally isolated from the human T-ALL cell line CEM. U-87 MG cells were infected with the recom-
binant retroviruses as described previously (17). Retrovirally infected pools were grown continuously in medium containing 400 μg/ml of G418 (Geneticin; Gibco-BRL, Grand Island, NY), and clonal transfectants were derived by limiting dilutional cloning.

**Analysis of Cell Death.** Apoptosis was quantitated using a multiparameter FACS assay (Becton Dickinson; Lysis II software, Lincoln Park, NJ) measuring forward light scatter and fluorescence of DNA-binding fluorochromes Hoechst 33342 (Sigma Chemical Co., St. Louis, MO) and propidium iodide, as described previously (18). Apoptosis was also quantitated by time-lapse video-microscopy using a Series 67 camera (DAGE-MTI, Inc., Michigan City, IN) and Panasonic NV-8500 time-lapse VCR at a final magnification of ×500. Cells were videotaped every minute for 4 days by taping them for 10 s under full illumination and allowing them to incubate for 50 s in the dark as described previously (19). For morphological assessment of chromatin structure, cells were seeded into glass chamber slides before irradiation. The effective dose of radiation received by cells on glass is approximately 40% higher than in other experiments using tissue culture plastic (20). One day after irradiation, the cells were fixed in 70% ethanol for 5 min, washed twice for 5 min each time with PBS, and stained with Hoechst 33342 (2 μg/ml in PBS) for 10 min at room temperature in the dark. Cells were then washed twice for 5 min in PBS and coverslipped, and 300 nuclei were examined by fluorescence microscopy. The net effect of both proliferation and cell death occurring in cultures was also analyzed by cell counting. Cells were either trypsinized before counting or fixed on tissue culture plates in 10% neutral buffered formalin, and 10 random, nonoverlapping fields (×100 magnification) were counted by microscopic examination for total cell number.

**Clonogenic Assays.** Cells were plated, grown, and irradiated as described above. On the day before irradiation, SF-126 cells were irradiated with 40 Gy and plated in 6-well plates to function as a feeder layer. On the following day, asynchronous cultures of cells the clonogenic survival of which was to be evaluated (Fig. 2A) were seeded as described previously (21). Briefly, after exposure to a specified dose of radiation, cultures were trypsinized with 0.05% trypsin, counted, and plated at specified concentrations into wells containing an irradiated feeder layer. For analysis of synchronized cells (Fig. 2, B and C), mitotic cells were irradiated 4 h or 15 h after plating into wells containing an irradiated feeder layer. All cultures were then incubated for 14–21 days, at which time colonies of over 50 cells were counted. Clonogenic assays were carried out in DMEM supplemented with 10% fetal bovine serum and 400 μg/ml of G418. Cell survival measurements were fitted to a linear quadratic mathematical model using the FIT program (Ver. 2.10; Ref. 22). The radiation dose sufficient to produce a 1 log cell kill and SEM was generated for each tested cell line using FIT software (Ver. 2.10; Ref. 22). The plating efficiency for all experiments was between 20 and 30%. In each independent experiment, 2–4 different dilutions were made per radiation dose, and each dilution was plated in multiples of six.

**Cell Cycle Analysis.** Beginning 16 h after irradiation, cells were labeled for 4 h with 10 mM BrdUrd (Sigma Chemical Co., St. Louis, MO), harvested by trypsinization, and washed with PBS. Cells (1 × 10^6) were then fixed in 70% ethanol at 4°C overnight and permeabilized with 0.1 M HCl and 0.5% Triton X-100 on ice for 10 min. Cells were then centrifuged, resuspended in 2 ml of distilled water, and boiled for 10 min. After a 1-h incubation with a fluorescein-conjugated anti-BrdUrd antibody (1:20 dilution; Boehringer Mannheim), cells were resuspended in 1.0 ml of PBS containing 50 mg/ml of RNase (Calbiochem, San Diego, CA) and 5 mg/ml of propidium iodide. Cells (10,000) were analyzed using a FACS (Becton Dickinson and Lysis II software, Lincoln Park, NJ). Only the first 2,000 cells analyzed are depicted in Fig. 2. Flow cytometry analysis allowed delineation of G1, cells that have a 2N DNA content and no BrdUrd incorporation, S-phase cells that have variable DNA contents and BrdUrd incorporation, and G2-M cells that have a 4N DNA content and no BrdUrd incorporation.

Labeling index was measured by seeding synchronized cells into 30-mm tissue culture dishes (20,000 cells/dish) under the culture conditions described above. The samples were incubated continuously beginning 2 h after irradiation treatment with low specific activity (5 Ci/mmol) tritiated thymidine at 1 μCi/ml. The cells were then washed twice with cold HBSS and fixed in 10% neutral buffered formalin for 30 min. After fixation, the cells were rinsed twice with 70% ethanol and air-dried before being coated with autoradiographic emulsion (Ilford, Warrington, PA). After a 7-day exposure, the emulsion was developed, and 500 cells from duplicate plates for each sample were scored as either labeled or unlabeled and averaged to generate one data point.

The mitotic index was measured by the squash technique (23). Briefly, 50,000 cells from each experimental sample were centrifuged at 4°C, fixed with 50% acetic acid, stained with 2% aceto-orcein, and then pressed under cover glass before microscopic examination. For each sample, 500 cells were scored for evidence of mitosis.

**p53 Protein Analysis.** p53 protein expression levels were determined by Western blotting. Cells were lysed on ice for 30 min in 100 ml of EBC buffer (50 mm Tris-HCl (pH 8.0)–120 mm NaCl–0.5% NP40–1 mm EDTA) containing leupeptin (10 μg/ml), aprotinin (10 μg/ml), phenylmethylylsulfonyl fluoride (1 mm), sodium o-vanadate (1 mm), and sodium fluoride (50 mm). Protein concentration was quantitated using the Bio-Rad Protein Assay according to the manufacturer’s instructions (Bio-Rad Laboratories, Richmond, CA). Total cell protein lysate was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and transferred to a nitrocellulose filter. The filter was blocked overnight with 5% nonfat milk in PBS containing 0.1% Tween 20 (PBS-T), probed for 1 h with 1 μg/ml anti-p53 antibody PAb 1801 (Oncogene Science, Inc., Uniondale, NY) in PBS-T, washed with PBS-T, and incubated with a 1:10,000 dilution of IgG conjugated to horseradish peroxidase in PBS-T (Sigma Chemical Co.). After further washes with PBS-T, the antibody reaction was detected using chemiluminescence detection (ECL; Amersham, Arlington Heights, IL) according to the manufacturer’s recommendations.

**Results.**

Influence of p53 function on cellular radiosensitivity cannot be detected in asynchronous cultures. The glioblastoma cell line U-87 MG expresses wild-type, functional p53 (11). To investigate the contribution of wild-type p53 to the radiation response of this cell line, we constructed isogenic clones of U-87 MG differing only in their p53 function. We inactivated wild-type p53 function in U-87 MG cells by introducing a dominant negative mutant p53 gene. U-87 MG cells were infected with a recombinant retrovirus encoding a p53 cDNA mutated at codon 175 or the LUX gene as a control. p53 protein expression was documented by Western analysis using an anti-p53 antibody that recognizes both the wild-type and mutant p53 proteins (data not shown). In a pool of numerous control vector transfectants (U87-LUX[pool]), p53 protein levels were inducible (4-fold) by 4 Gy of ionizing radiation. In contrast, U-87 MG cells transfected with a vector encoding a mutant p53 (U87–175[pool]) showed high levels of p53 expression (40-fold greater than in nonirradiated U87-LUX cultures) that were unchanged by radiation exposure. Individual clonal isolates of the two transfected pools were derived by dilutional cloning for further analysis.

We documented that expression of the dominant negative mutant p53 in U-87 MG inactivated the function of endogenous wild-type p53 (Fig. 1). An examination of cell cycle distribution by FACS analysis demonstrated a radiation-induced G2 arrest in the parental U-87 MG cells (Fig. 1, A and B) and also in a clonal isolate transfected with control vector U87-LUX.8 (Fig. 1, E and F), as well as U87-LUX[pool] and another clonal isolate U87-LUX.4 (data summarized below). These cells exhibited an accumulation of cells in G1 and a depletion of the S-phase population 16 h after exposure to 4 Gy of ionizing radiation, which has been attributed to an arrest of cells in G1 mediated by wild-type p53 (10). Radiation-induced changes in cell cycle distribution indicative of a G1 arrest were absent in four clonal isolates expressing dominant negative mutant p53, U87–175.4 (Fig. 1, B and C), U87–175.2, U87–175.6, and U87–175.13, as well as U87–175[pool] (data summarized below). To quantitate the depletion of the S-phase fraction 16 h after irradiation, we compared the fraction of S-phase cells, as determined by the FACS analysis depicted in Fig. 1, in irradiated and nonirradiated cultures. After irradiation of cells with a functional p53, the S-phase fraction was reduced by 82–92%.
In contrast, the S-phase fraction of cells in which wild-type p53 was inactivated was reduced by only 21–58%.

To further characterize the p53-dependent G1 arrest that followed irradiation of U87-LUX.8 cells that express only wild-type p53, we irradiated these cells with 20 Gy and evaluated them for 10 weeks. No change in cell number and no signs of cell death, such as abnormal cellular morphology or cellular debris in the medium, were apparent during the observation period (data not shown). FACS analysis of the cell cycle distribution at the end of the 10-week observation period demonstrated this culture to be in G1 (data not shown). These data are consistent with the possibility that p53 mediates an irreversible G1 arrest of these cells after irradiation.

We used clonogenic survival assays to assess the effect of p53 inactivation on the radiosensitivity of the isogenic clones described above. Expression of dominant negative mutant p53 was not associated with a significant change in survival after irradiation (Fig. 2A). The radiation doses sufficient to reduce cellular clonogenicity of asynchronous U87-LUX.4 (control vector), U87-LUX.8 (control vector), and U87–175.4 (dominant negative mutant p53) cells by 1 log were 3.85 ± 0.17 Gy, 3.73 ± 0.19 Gy, and 3.98 ± 0.30 Gy, respectively.

We also examined whether inactivation of wild-type p53 was associated with altered susceptibility of glioblastoma cells to radiation-induced apoptosis. The clonal isolates U87-LUX.8 and U87-LUX.6, expressing only endogenous wild-type p53, and U87–175.4, expressing a dominant negative mutant p53, were assayed for apoptosis one day after exposure to 4 Gy of ionizing radiation using a multiparameter FACS analysis. No significant apoptosis was evident in any of the cells examined (data not shown). The minimal apoptotic response after irradiation of these clones was confirmed in other studies examining cells at several different times after various doses of ionizing radiation by a terminal deoxynucleotidyl transferase-labeling technique.  

**Inactivation of p53 Function Results in Radioreistance of Synchronous Cultures.** To evaluate more critically the effect of the p53 on the clonogenic survival of U-87 MG cells, we sought to synchronize cells in a manner that would allow us to irradiate cultures of cells synchronized in G1. Transfected clonal isolates of U-87 MG were synchronized in the cell cycle by mitotic selection, yielding a highly homogeneous population of cells (93–96% in mitosis). Within 4 h of plating, the mitotic cells attached to the culture plates, completed cell division, and assumed a morphology typical of interphase U-87 MG cells (data not shown). At this time, synchronous cell cultures were treated with 4 Gy of ionizing radiation. Irradiated and nonirradiated populations were then incubated continuously with tritiated thymidine to assess their passage through G1 and into S phase. S-phase nuclei of these synchronized cells were scored for thymidine incorporation at 7, 15, 19, 24, 36, and 48 h after plating (Fig. 3). U-87 MG cells transfected with a control vector (U87–LUX.8) exhibited a G1 arrest after irradiation. When examined 24 h after plating, 60% of the nonirradiated cells had entered S phase, as evidenced by incorporation of thymidine, whereas only 3% of the irradiated cells had entered S phase, indicating a 95% inhibition of cell cycle progression (Fig. 3A). Greater than 90% of the cells that manifest the p53-mediated G1 arrest in the first postirradiation cell cycle do not reenter the cycle for at least 2 days (Fig. 3A). In contrast, almost no inhibition of cell cycle progression was detectable in the G1-synchronized clones expressing mutant p53 (Fig. 3B). The experiment was repeated using higher doses of ionizing radiation (6 and 9 Gy), and similar results were obtained (data not shown).

The total number of cells in parallel cultures of U87-LUX.8 (control vector) and the U87–175.4 (dominant negative mutant p53) cells were counted at several time points after irradiation in early G1. Forty-eight h after exposure to 4 Gy of ionizing radiation, the total number of U87-LUX.8 cells did not change, whereas the total number of U87–175.4 cells nearly doubled, indicating that the transfected derivative expressing a dominant negative mutant p53 continued to proliferate (data not shown). Another experiment was conducted with U87-LUX.8 cells irradiated in early G1 and followed for 6 days. No change in total cell number was seen in cultures irradiated with 6 Gy, whereas parallel, nonirradiated cultures grew to confluence (data not shown). These data also provide evidence compatible with our finding that the U-87 MG cells expressing wild-type p53 do not undergo extensive apoptosis, because the total cell number remains unchanged.

Clonogenic survival assays were performed on synchronized cells that were irradiated in early G1 to assess more sensitively the effect of p53 inactivation on radiosensitivity. Cells synchronized by mitotic selection were seeded into cell culture plates and allowed 4 h to progress into early G1 before irradiation. When synchronous cells were irradiated in early G1, a marked increase in clonogenic survival was seen in transfecants lacking functional p53 (Fig. 2B). The radiation doses sufficient to reduce cellular clonogenicity by 1 log in...
synchronized U87-LUX.8 and U87-175.4 cells irradiated in early G1 were 3.26 ± 0.12 Gy and 7.41 ± 0.43 Gy, respectively.

Microscopic examination of chromatin morphology was used to evaluate the occurrence of apoptosis in synchronous glioblastoma cells exposed to a high dose of radiation in early G1. U87-LUX.8 (control vector) and U87-175.4 (dominant negative mutant p53) cells were synchronized by mitotic selection, plated onto glass chamber slides, and treated 4 h later with 0 or 20 Gy of ionizing radiation. Cells were fixed 1 day after irradiation, and their nuclei were stained with the DNA-binding fluorochrome Hoechst 33342. Cells were examined by fluorescence microscopy in two experiments for characteristics of apoptosis, such as condensed nuclei and fragmented chromatin. No evidence of apoptosis was observed (data not shown). The nuclei of nonirradiated and irradiated cells from both cell lines were large and stained uniformly.

We used time-lapse videomicroscopy to observe U-87 MG cells expressing wild-type p53 after radiation treatment during G1 of synchronous cultures (Fig. 4). Mitotic U87-LUX.8 cells were seeded into tissue culture flasks and allowed 4 h to progress into early G1. At that time, cells were treated with 0 or 4 Gy of ionizing radiation, and individual cells were followed by time-lapse videomicroscopy for 4 days. Both the control cells (0 Gy) and the irradiated cells exhibited low levels of cell death (3% and 0%, respectively; data not shown). Seventy-five % of control cells entered mitosis and divided, which is consistent with labeling index data from parallel cultures showing that 68% of control cells progressed through the G1-S phase transition (data not shown). In contrast, none of the cells irradiated with 4 Gy divided during the observation period. Labeling index data collected 24 h after irradiation confirmed that irradiated cells were arrested in G1 (approximately 5% labeled; data not shown).

Clonogenic survival assays were performed on U87-LUX.8 cells synchronized in early versus late G1 to further assess the effect of the p53-mediated G1 arrest on radiosensitivity. The p53-dependent G1 arrest that occurs after irradiation of normal human diploid fibroblasts is not observed in cells that have progressed into late G1 (24, 25). Cells synchronized by mitotic selection were seeded into cell culture plates and allowed 4 h to progress to early G1 or 15 h to progress to a late stage of G1, at which times they were irradiated. The degree of cell cycle synchrony decreases at later time points after mitotic selection (data not shown); however, an increase in clonogenic survival was detected in U87-LUX.8 cells irradiated 15 h after mitotic selection, as compared to U87-LUX.8 cells irradiated 4 h after mitotic selection (Fig. 2C). The radiation doses sufficient to reduce cellular clonogenicity by one log in early G1-synchronized cells was 3.15 ± 0.08 Gy, whereas 4.17 ± 0.20 Gy was required to achieve the same killing effect in cells irradiated during late G1.

Discussion

Recent discoveries indicating that p53 can mediate a G1 cell cycle arrest and apoptosis in cells exposed to radiation have contributed significantly to our understanding of the cellular response to radiation (2). The relationship between p53 gene expression and cellular radiosensitivity, however, is unclear. Increased radiosensitivity, decreased radiosensitivity, and no apparent difference in radiosensitivity have all been associated with p53 function in various studies (26–28). We were initially unable to detect any significant difference in radiosensitivity between isogenic glioblastoma cell lines differing only in the functionality of p53 (Fig. 2A), despite a strong dependence of the radiation-induced G1 arrest in these cells on p53 (Fig. 1). These results
prompted us to devise an experimental strategy to look more critically at the influence of p53 function on radiosensitivity.

The radiosensitivity of cells is affected by their position in the cell cycle at the time of irradiation (29). Typically, asynchronous cultures consisting of cells in various phases of the cell cycle are used for studies of radiosensitivity. When such cultures are irradiated, cells in different phases of the cell cycle respond differently (30). In experiments analyzing asynchronous cultures of cells, radiosensitivity is generally dominated by S-phase cells because these cells are more resistant to the lethal effects of ionizing radiation (31). The p53-dependent G1 arrest that occurs after irradiation of normal human diploid fibroblasts is not observed in cells that have progressed into late G1 (24, 25). Thus, the influence of the p53-mediated G1 arrest on radiosensitivity could be masked in experiments using asynchronous cultures of cells. Using cultures of synchronized cells that were irradiated in early G1, we found that inactivation of p53 results in a significant decrease in radiosensitivity (Fig. 2B).

Our finding that loss of p53 function decreases the sensitivity of glioblastoma cells to irradiation is consistent with several studies of other human cell types; however, the mechanism underlying the influence of p53 on radiosensitivity seems to vary among cells of different lineages. Radiation-induced DNA damage can trigger p53-dependent apoptosis in various cell types, and loss of the apoptotic response decreases the radiosensitivity of murine thymocytes and hematopoietic cells (32). Our observation that human glioblastoma cells and isogenic derivatives in which p53 function had been inactivated have similar apoptotic responses to irradiation rules out p53-mediated apoptosis as an important determinant of radiosensitivity in these cells. Glioblastoma cells expressing functional p53 arrested in the first postirradiation G1 and remained blocked in this phase of the cell cycle for several days (Figs. 3 and 4). The prolonged duration of the p53-dependent G1 arrest after irradiation suggests that p53 may eliminate radiation-damaged cells from a population by mediating their irreversible arrest in G1. Such an arrest is a likely mechanism of reproductive failure, as is suggested by studies of human diploid fibroblasts (25, 33). Thus, the radioresistance we observed in tumor cells lacking a functional p53 may result, in part, from a loss of the prolonged G1 arrest that occurs in cells expressing a wild-type p53. This hypothesis is supported by our finding that glioblastoma cells expressing a functional p53 were more radioresistant when irradiated during late G1 than during early G1 (Fig. 2C).

Further investigation of glioblastoma cells expressing functional p53 is required to determine the characteristics of the clonogenic cells observed after irradiation during early G1. The relatively low survival that was observed for the U87-LUX.8 cells (Fig. 2, B and C) may be due to cells irradiated in G1 that ultimately recovered from the G1 arrest. Alternatively, the clonogenic cells may originate from contaminating cells (4—7% of the irradiated population) that were not in G1 and possibly in the relatively radioresistant S phase. A third possibility is that a fraction of the U87-LUX.8 cells irradiated in early G1 acquired mutations that inactivate the p53 signal transduction pathway. In any case, the cells irradiated in early G1 were much more radioresistant when they expressed functional p53 than when they did not.

Our finding that glioblastoma cells expressing functional p53 arrested in the first postirradiation G1 for several days was unexpected, because reproductive failure after irradiation is thought to be triggered by chromosomal aberrations that develop after irradiated cells complete the first postirradiation cell cycle and undergo one or many subsequent mitotic cycles (3, 34). Our finding is consistent with the recently published observation in another glioblastoma cell line that conditional expression of wild-type p53 protein can cause a G1 arrest during the first cell cycle when p53 levels are experimentally elevated (35). However, the only previous analysis of human tumor cells...
exhibiting a radiation-induced G1 arrest in which the cell cycle was analyzed critically determined that tumor cells differ from normal cells in that they arrest in the second postirradiation cell cycle (36). These investigators hypothesized that tumor cells had lost the G1 checkpoint that stops cell cycle progression of normal cells with damaged DNA. Our finding of a prolonged radiation-induced G1 arrest during the first postirradiation cell cycle of U-87 MG glioblastoma cells does not support this hypothesis. The significance of which cell cycle is perturbed by radiation treatment is critical for the development of a testable hypothesis regarding the molecular mechanisms mediating the response of both normal and tumor cells to radiation. For example, the molecular signals that initiate cell death pathways may vary greatly before and after the cell has completed the complex process of mitosis. Future experimentation considering the distribution of cells in the cell cycle at the time of irradiation may clarify the complex issue of how the many functions mediated by p53, including cell cycle arrest (10), the modulation of apoptosis (4), and DNA repair (37), contribute to the cellular response to radiation.

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